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The efficacy of topically applied Sappan wood (*Caesalpinia sappan* L.) ethanol extract during incision wound healing in albino rats

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Abstract

Background: Medical manifestations in the form of incisions, burns, and trauma will trigger a natural wound-healing process that involves complex interactions between cells. Brazilin and other secondary metabolites found in Sappan wood have numerous bioactive qualities, including anti-inflammatory, anti-cancer, and antioxidant properties.

Aim: This study aimed to investigate the efficacy of Sappan wood (*Caesalpinia Sappan* L.) ethanol extract topically on the incision wound healing of albino rats.

Methods: Twenty male rats were randomly assigned into five groups with four replications, i.e., (C-) was treated ointment-based, (C+) was treated with 10% povidone-iodine, (T1, T2, and T3 groups) were treated with Sappan wood extract concentration for 6.5%, 15%, and 30%, respectively. The treatment was topically administered to wounded areas twice a day for 15 days. Wound healing was evaluated histologically as the following parameters collagen deposition, polymorphonuclear neutrophils (PMN), angiogenesis, and fibrosis degree using H&E staining. IL-2 level was evaluated using the enzyme-linked immunosorbent assay (ELISA) method. Wound length reduction was calculated on days 8 and 15.

Results: As a result, the 6.5% (T1), 15% (T2), and 30% (T3) Sappan wood extract groups were improved significantly ($p < 0.05$) compared to ointment-based (C-) and povidone-iodine (C+) groups on the collagen deposition, PMN, angiogenesis, fibrosis degree, and IL-2 level. In particular, the 6.5% (T1) Sappan wood extract group was highlighted significantly ($p < 0.05$) compared to other groups, evidenced by the improvisation of wound healing parameters and reduction of wound length on days 8 and 15.

Conclusion: In conclusion, a 6.5% Sappan wood extract revealed its applicability to improve incision wound healing in albino rats.

Keywords: Drug safety, Incision wound, Sappan wood, Wound healing process.

Introduction

A wound is an anomaly on the skin caused by injury to tissue units or components. Accidental skin wounds can result from accidents or from cuts and scratches to the skin. A wound may also be intentionally caused, for example, by making an incision wound for a surgical procedure. Wounds typically result in discomfort, bleeding, incapacity, and difficulties during therapeutic procedures (Stanley, 2017).

The wound healing process is complicated because it involves multiple interconnected phases, including phases of inflammation, proliferation, and remodeling. An essential element in the healing of wounds is collagen (Triana *et al*., 2020). Collagen has a role in wound healing through its capacity to maintain homeostasis and interact with fibronectin and platelets. Platelet aggregation and activation resulting from the contact of fibrillar collagen with blood would release chemotaxis factors, initiating the wound healing process (Hosseinzadegan and Tafti, 2017). Moreover, collagen may accelerate the fibroplasia process, cellular components, growth factors, and fluid exudation (El-Hamoly *et al*., 2019).

The most popular method for promoting wound healing is 10% povidone-iodine. Povidone-iodine was selected as the most preferred treatment due to its antibacterial action, ability to produce angiogenesis, and ability to reduce inflammation (Bigliardi *et al*., 2017). In vitro

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studies suggested that the use of povidone-iodine as a wound-healing treatment was debatable due to the possibility of side effects (Punjataewakupt *et al*., 2019). Povidone-iodine may also prevent fibroblast growth since fibroblast migration and proliferation in the injured area are factors in wound healing. Fibroblast proliferation controls the healing outcome of the wound by influencing the re-epithelialization process, which closes the wound, and produces collagen. The potential for wound healing may be compromised by a slowed rate of fibroblast proliferation (Rodrigues *et al*., 2019). The use of herbal remedies, for example, is also favored more because of its low cost and high degree of safety. Numerous natural resources, including Sappan wood (*Caesalpinia sappan* L.), may be found in the tropical nation of Indonesia. The Sappan wood is a member of the Leguminosae family, also known as Brazilian wood in general. The biological effects of Sappan wood include anti-inflammatory, antibacterial, antioxidant activity, allergy, nuclease activity, and analgesia (Vij *et al*., 2023). Flavonoids, saponins, alkaloids, tannins, phenolics, and brazilin were the secondary metabolites that were active in Sappan wood. Alkaloids, saponins, and flavonoids have anti-inflammatory, antifungal, and antioxidant effects. Tannins also have the potential to produce antiviral and antibacterial activity. Brazilin, a particular chemical found in Sappan wood, has anti-inflammatory properties (Ahmad *et al*., 2021). Based on the scientific evidence literature, Sappan wood, which is widely accepted, offers therapeutic benefits. The primary objective could suggest that the heartwood of Sappan wood has a great deal of therapeutic potential. It is recommended to utilize wood rather than logwood when using medication. It is widely utilized in Unani and Ayurvedic therapy. Brazilin has a high level of latent pharmacological activity, healing, anticancer, antidiabetic, and immunostimulant qualities. Numerous studies have demonstrated the many health benefits of sappan wood, although the precise mechanism underlying these benefits is still unclear (Vij *et al*., 2023). As previously explained, an investigation in this study was required to determine the effectiveness of Sappan wood on incision wound healing in albino rats.

Materials and Methods

Animals

The Federer formula was used to calculate the sample size for each group: $t(n–1) \geq 15$, yielding $n \geq 4$; here, t represents the number of groups and n denotes the replication sample size. For each group in this investigation, a minimum sample size of four animals was used. This formula design was taken into consideration because of two related factors: replication and randomization are required in order to provide a reliable estimate of the error variance of a contrast. A total of 20 male, healthy, and deformity-free albino rats were included in this study, then divided into five treatment groups with four replications, i.e., (C-) treated with ointment-based, (C+) treated with 10% of povidone-iodine, (T1) treated with 6.5% of Sappan wood extract, (T2) treated with 15% of Sappan wood extract, and (T3) treated with 30% of Sappan wood extract, respectively.

Topical ointment preparation

Sappan woods were collected, washed, and allowed to dry under direct sunlight exposure for two days. They were then blended and sieved through a strainer with an 80-mesh diameter. A total of 2000 g of the powder product was kept in a container that was securely closed and then submerged in a 95% ethanol solution under reflux circumstances for three days at 27°C to continue achieving these properties. After that, the filtrate of the ethanol extract was concentrated using a rotary evaporator until viscous semi-solid masses were the result. The concentrations of 6.5%, 15%, and 30% as they relate to in vitro wound healing performed in these treatment groups. The fusing process was used to make the ointment bases, which were hydrophilic petrolatum (HP), absorptive ointment (AO), and hydrophilic ointment (HO). Using an ointment spatula, the ointment bases and Sappan wood extract were homogenized on a ceramic slab as part of the method. The ointment base was mixed in the same method after the hydrophilic polymer was added. After being further combined in a water bath that had been heated to 80°C, the kneaded mixture was allowed to cool at 27°C.

Incision and treatment procedure

To simplify the incision process, the rats in all groups had their paravertebral region, measuring 3 × 2.5 cm, shaved off. The rats were sedated beforehand with ketamine and diazepam (100 and 5 mg/ml) at a dose of 1 ml/kg BW before the creation of the wound. The incision was then made in the area that had been shaved, measuring 3 × 0.25 × 0.01 cm, directly toward the caudals (Yuniarti *et al*., 2018). Subsequently, a cotton bud was used to apply Sappan wood extract in ointment based on certain groups and Betadine® ointment (Batch No. PKD20501710078, Mundipharma Healthcare, Indonesia) to others as a topical treatment. During 8 and 15 days, those treatments were administered twice a day with a 12-hours gap. Following their ether-per-inhalation sacrifice, the albino rats' skin tissue and blood were collected. Using a scalpel, skin tissue was removed from the top, bottom, right, and left sides of the incised wound at a depth of 0.5 cm and more than 1 cm to prepare the tissue for histology.

Postoperative care

During the 15 days of the study, each rat was provided with a diet that was appropriate for meals and regular access to water while being kept individually in standard laboratory cages. During the study, clinical signs at the spot of the incision were monitored every day to assess any irregularities or possible infections. On days 8 and 15, the reduction in incision length was assessed using the vernier caliper in centimeters (cm).

Histopathological evaluation

The following was the definition of the histologic criteria for wound healing evaluation, i.e., A) Collagen deposition was scored (2) Normal collagen bundles, (1) unorganized/edematous collagen, and (0) amorphous collagen; B) Polymorphonuclear neutrophils (PMN) were scored (2) PMN cell counts of 0–10, (1) 1–40, and (0) >40; C) Angiogenesis degree was categorized into mild, moderate, and severe; D) Fibrosis degree was categorized into mild, moderate, and severe (Yuniarti *et al*., 2018).

Evaluation of IL-2 by ELISA

For the measurement of IL-2, commercial enzyme-linked immunosorbent assay (ELISA) kits were used (Endogen, Inc, USA). The usage of the ELISA kits was done in compliance with the handbooks. The ELISA kit's lower detection limits were 6 pg/ml.

Statistical analysis

The format for all the data was mean ± standard deviation. The Kolmogorov–Smirnov normality test was implemented to examine the distribution of the data. Analysis of Variance (one-way ANOVA) was used to investigate continuous variables with parametric distributions. If the findings were significant, a post hoc Duncan's comparison test was carried out. The Kruskal-Wallis and post hoc Mann-Whitney tests were used for multiple comparisons on data having a non-parametric distribution. The statistical program SPSS (IBM, USA) was used for each analysis. P values < 0.05 were considered as significant.

Ethical approval

The current study was authorized by The Ethical and Health Research Committee, Universitas Airlangga with reference No. 452/HRECC.FODM/VII/2021. This ethical approval was considered to avoid animal abuse during the study.

Results

Wound length evaluation

There were no adverse reactions on the animals or the area of the wound during the investigation. At postoperative days 8 and 15, the wound healing process was clinically unremarkable in all groups. The reduction in wound length was reported to be significant in the T1 group compared to the other groups ($p < 0.05$) on days 8 and 15 (Table 1).

Histopathological findings

In general, the T1 group was found to be significantly different from C-, C+, T2, and T3 groups ($p < 0.05$) on the wound healing parameters, i.e., collagen, PMN, angiogenesis, and fibrosis degree. This study's findings also indicated that the treatment groups T1, T2, and T3 reported significant compared to C+ ($p < 0.05$) (Table 2). In comparison to other groups, the C- group had a lower collagen deposition, closed incision wound region, angiogenesis, and PMN infiltration (Fig. 1). Compared to the T1 and T2 groups, the histological figure of the T3 group indicated less commonly generated collagen tissue and fibrosis degree (Figs. 1 and 2). Briefly, the group treated with 6.5% Sappan wood extract (T1) showed increased collagen synthesis, angiogenesis, and tissue fibrosis, all of which pointed to a favorable healing phase.

Evaluation of IL-2 cytokine levels

The T1, T2, and T3 groups were reported the highest blood serum IL-2 levels on day 15. However, the ointment with 6.5% Sappan wood extract used in T1 was improved significantly for wound healing compared to other groups ($p < 0.05$) (Table 2).

Discussion

In this study, the C- group reported the lowest collagen deposition, presence of PMN, angiogenesis, fibrosis

Table 1. Average wound length reduction on days 8 and 15.

Treatment groups	Length reduction (cm)	
	Day 8	Day 15
C-	0.48 ± 0.96^{d}	1.78 ± 0.15^{c}
C+	1.18 ± 0.13^{b}	2.38 ± 0.09^{ab}
T1	1.35 ± 0.13^{a}	2.58 ± 0.09^{a}
T2	1.00 ± 0.08^{c}	2.20 ± 0.23^{b}
T3	0.98 ± 0.13^{c}	2.30 ± 0.08^{b}

Values were expressed in the mean ± SD. [a,b,c,d] Different superscripts on the same column indicated a significant difference ($p < 0.05$).

Table 2. Evaluation of the wound healing process on day 15.

Treatment groups	Collagen	PMN	Angiogenesis	Fibrosis	IL-2 (ng/l)
C-	0.9 ± 0.19^{c}	1.0 ± 0.16^{c}	1.7 ± 0.38^{c}	1.8 ± 0.40^{c}	76.10 ± 0.76^{e}
C+	1.4 ± 0.16^{b}	1.4 ± 0.19^{b}	2.4 ± 0.44^{ab}	2.5 ± 0.58^{ab}	100.15 ± 0.95^{b}
T1	1.7 ± 0.26^{a}	1.8 ± 0.25^{a}	2.8 ± 0.40^{a}	2.8 ± 0.40^{a}	106.10 ± 1.27^{a}
T2	1.4 ± 0.19^{b}	1.6 ± 0.25^{ab}	1.9 ± 0.25^{bc}	2.0 ± 0.00^{bc}	95.93 ± 0.69^{c}
T3	1.4 ± 0.00^{b}	1.6 ± 0.10^{ab}	2.1 ± 0.19^{bc}	1.9 ± 0.38^{bc}	94.10 ± 0.32^{d}

Values were expressed in the mean ± SD. [a,b,c,d] Different superscripts on the same column indicated a significant difference ($p < 0.05$). PMN= Polymorphonuclear neutrophils.

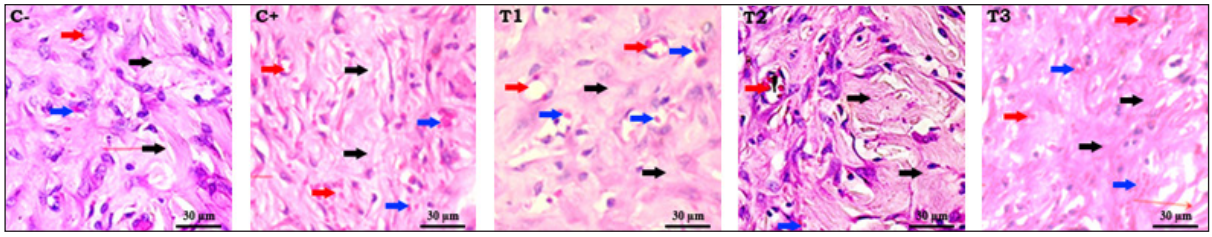

Fig. 1. Histology of (Black arrows) collagen deposition, (Blue arrows) PMN score, and (Red arrows) angiogenesis degree in all treatment groups on day 15 (Hematoxylin and Eosin, 400×).

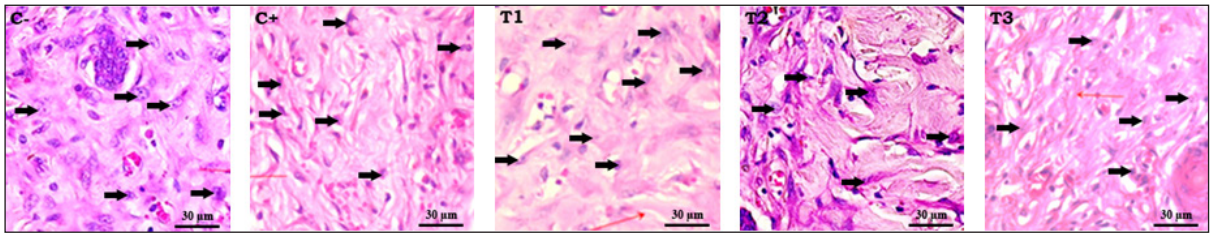

Fig. 2. Histology of (Black arrows) fibrosis degree in all treatment groups on day 15 (Hematoxylin and Eosin, 400×).

degree, and IL-2 level. The C-group of albino rats with incision wounds received no compounds or medications that would have aided in the rats' wound-healing process, which is how the outcome was reached. The insufficient presence of advantageous substances or medications resulted in extended inflammation, inappropriate wound healing, and sluggish collagen stimulation (El-Hamoly *et al*., 2019).

This study revealed that the wound healing parameters of the C+ group were comparable to the T3 group and lessen than those of other treatment groups. Povidone-iodine was administered to the C+ group, which included antibacterial components, and the outcome was observed. Antibacterial ingredients stop microbial development and contamination by inhibiting protein production, which in turn aids in the healing process of wounds. By reducing excessive inflammation, this medication may also result in an increase in collagen deposition and accelerate the healing of wounds during the inflammatory phase (Cunha *et al*., 2019).

The T1 group showed a significant improvement ($p < 0.05$) in fibrosis degree, angiogenesis, PMN presence, collagen deposition, and IL-2 compared to the other treatment groups. This study demonstrated that the ideal dosage for Sappan wood extract ointment was 6.5%. These outcomes had to do with the Sappan wood secondary metabolites, which had therapeutic value. Among the secondary metabolites found in plants, especially in Sappan wood, are flavonoids, saponins, tannins, alkaloids, and specific active compounds like brazilin (Vij *et al*., 2023).

The bacterial peptidoglycan membrane becomes more permeable when saponins contact with bacterial cells, causing the bacteria to be lysed (Fikri *et al*., 2020). Saponins have the potential to enhance monocyte proliferation by increasing the quantity of macrophages and secreting growth factors that aid in the production of fibroblasts and the synthesis of collagen in the wound area (Solikhah *et al*., 2022). Additionally, saponins could quicken keratinocyte migration, which was crucial to the re-epithelialization process (Shin *et al*., 2018). Flavonoids possess the capacity to lower free radicals as an antioxidant. Free radicals have the potential to impede wound healing by suppressing collagen tissue contraction, inflammatory responses, and cell proliferation (Engwa, 2018).

Several substances found in Sappan wood, such as brazilin, have antibacterial and anti-inflammatory properties. Due to its greater antioxidant efficacy compared to commercial antioxidants like BHT and BHA, betelain found in Sappan wood has greater promise as a free radical counter-agent (Fasihnia *et al*., 2020). Antioxidants can bind to free radicals and minimize damage to cell membranes during the proliferative phase (Acworth *et al*., 2017). Because the phenolic compounds in Sappan wood stopped the chain reaction caused by the emergence of free radicals, flavonoids, and brazilin were also able to halt oxidation reactions. As hydrogen donors, phenolic substances can inhibit the production of free radicals (Zeb, 2020). The tannin component in Sappan wood extract may promote wound healing by several cellular processes, including removing reactive oxygen species and free radicals, promoting wound grafting, and stimulating capillary blood vessels and fibroblasts. Tannins served as astringents, preventing minor bleeding and exudate to hasten the healing process (Medel-Marabolí *et al*., 2017). Fibroblast migration and proliferation in the wound area were influenced by tannins and saponins (Rodrigues *et al*., 2019).

Humidity was another element that could vary and have an impact on the outcomes. Because the moisture was occlusive to water and germs yet permeable to oxygen and water vapor, wound healing proceeded unhindered (Panggabean *et al*., 2023). One of the most crucial components that affect how cells metabolize energy and heal wounds through oxygenation responses is oxygen (de Smet *et al*., 2017). Compared to the T2 and T3 groups, the T1 group had a higher moisture content. Elevated oxygen levels in the wound tissue could be caused by sufficiently high moisture levels. Moreover, collagen production may be accelerated and stimulated by the fibroblast proliferation process. Oxygenation and moisture content had an impact on the outcome, as they were two of the key elements affecting the wound healing process (Gupta *et al*., 2022). Reduced oxygen pressure in wound tissue would be the result of reduced moisture content. Reduced oxygen pressure might impact fibroblast, macrophage, and neutrophil function, leading to a small inhibition of collagen formation (Wlaschek *et al*., 2019).

The three stages of the wound healing process are generally referred to as the inflammatory, proliferation, and remodeling phases. Proliferating macrophages gather in the vicinity of the wound during the inflammatory phase (Hisyam *et al*., 2023). Conversely, the proliferative phase will be accompanied by the development of collagen, angiogenesis, and epithelialization (Prastika *et al*., 2020). In the following remodeling phase, fibroblasts, collagen, edema, and new blood vessels will all rapidly form and mature. In order to keep the tissue strong around the wound, collagen is essential (Hamid *et al*., 2018; Spielman *et al*., 2023). Put another way, since Sappan woods have antibacterial, anti-inflammatory, and antioxidant properties, giving those rats 6.5% Sappan wood extracts standardized to ointment-based may hasten the healing of the incised wound relative to other treatment groups. The process of wound healing will involve the synergy of these three actions.

Thus, our findings supported the following study hypothesis that Sappan wood contains active chemicals that promote wound healing, particularly when used topically as an ointment. In addition to being an angiogenic agent, the fibroblast growth factor can also bind to its receptor and form a complex with heparin, which increases the latter's cell activation effectiveness (Zulueta *et al*., 2018). The fibroblast population growth helps quicken the healing of wounds. Three groups of angiogenic factors can be distinguished as agents that accelerate mitosis by targeting endothelial cells making up the first group (Eelen *et al*., 2020). The second category consists of compounds that, aside from endothelial cells, activate a wide variety of target cells. This group includes several chemokines, cytokines, and angiogenic enzymes (Lesmana *et al*., 2023). The cytokine from this group that was initially described was fibroblast growth factor 2 (Rani *et al*., 2022). Indirect factors make up the third category. Macrophages and endothelial cells are the producers of these angiogenic factors. Pathological and physiological processes involve angiogenesis (Al-Anshori *et al*., 2023). Angiogenesis is a naturally occurring, highly regulated physiological process that is essential to the healing of wounds in a healthy body (Ramirez-Pedraza and Fernández, 2019).

Moreover, Sappan wood can lessen protein translation and polyethylene glycol-2. Additionally, they efficiently lower inflammatory cytokine levels, including IL-1, IL-6, and TNF-α (Haryanti *et al*., 2018). Lymphocyte cells produce soluble protein cytokines, such as IL-2, which serve as intracellular signals and control almost every aspect of cell life, including growth, proliferation, and differentiation (Kartikasari *et al*., 2020). IL-2 is a cell growth factor that controls the proportion of lymphocytes that are activated. Th-1 cells normally release IL-2 upon their activation by mitogens or antigens. Nevertheless, secondary messengers are necessary for optimum production (Zhu, 2018). These results reported that the levels of the cytokine IL-2 varied significantly throughout the treatment groups. This investigation revealed that gels containing different doses of Sappan wood extract significantly raised the levels of IL-2 in the blood serum of albino rats (Tukiran *et al*., 2023). In rats with iron overload, sappan wood ethanol extract can also raise total iron binding capacity (TIBC) and transferrin concentration while lowering ferritin levels and transferrin saturation. The ethanol extract from sappan wood may be utilized as an adjuvant and an alternative to iron chelators (Pitaloka *et al*., 2022). A potential limitation of this study could be the necessity to investigate the degree to which clinical trials can be utilized to replicate human use. To fully understand the wound status, more analysis of the epithelialization period data is required. On the other hand, no signs of an infection that would have slowed down the healing process were observed.

Conclusion

In conclusion, applying topically Sappan wood extract to albino rats' incision wounds for 15 days can improve collagen deposition, PMN, angiogenesis, fibrosis degree, and IL-2 level. In particular, applying a 6.5% topical concentration of Sappan wood extract revealed significant efficacy in the reduction of length and wound healing parameters. Further clinical studies are required to investigate the epithelialization period and particular phases of wound healing using electron microscopy.

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Conflict of interest

The authors declare that they have no competing interests.

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Authors contribution

FF contributed to conceptualization, methodology, funding acquisition, and supervision. RES, AP, and STM contributed to investigation, prepared extraction, treated animals, and wound healing evaluation. STM, FF, and MTEP contributed to project administration, visualization, validation, data curation, writing-original draft, and editing. All authors have read and approved the final manuscript.

Data availability

All data are provided in the manuscript.

References

Acworth, I.N., McCabe, D.R. and Maher, T.J. 2017. The analysis of free radicals, their reaction products, and antioxidants. In Oxidants, antioxidants and free radicals. Boca Raton, FL; CRC Press, pp: 23–77.

Ahmad, I., Setyaningsih, E.P., Arifianti, A.E., Saputri, F.C. and Munâ, A. 2021. Optimization of ionic liquid-based microwave-assisted extraction on brazilin levels from sappan wood and its dipeptidyl peptidase IV inhibition activity. J. Appl. Pharm. Sci. 11(7), 072–079.

Al-Anshori, A.A., Putri, A.N., Ismi, A.N., Suhud, M.K., Plumeriastuti, H. and Maslachah, L. 2023. Efficacy of transdermal delivery nano ethosomal gel from ashitaba leaves on *in-vivo* burn wound healing in albino rats. J. Med. Vet. 6(2), 145–154.

Bigliardi, P.L., Alsagoff, S.A.L., El-Kafrawi, H.Y., Pyon, J.K., Wa, C.T.C. and Villa, M.A. 2017. Povidone iodine in wound healing: a review of current concepts and practices. Int. J. Surg. 44(1), 260–268.

Cunha, J.L.S., Carvalho, F.M.D.A.D., Pereira Filho, R.N. and Ribeiro, M.A.G. 2019. Effects of different protocols of low-level laser therapy on collagen deposition in wound healing. Braz. Dent. J. 30(1), 317–324.

de Smet, G.H., Kroese, L.F., Menon, A.G., Jeekel, J., van Pelt, A.W., Kleinrensink, G.J. and Lange, J.F. 2017. Oxygen therapies and their effects on wound healing. Wound Repair Regen. 25(4), 591–608.

Eelen, G., Treps, L., Li, X. and Carmeliet, P. 2020. Basic and therapeutic aspects of angiogenesis updated. Circ. Res. 127(2), 310–329.

El-Hamoly, T., Abd El-Rahman, S.S. and Al-Abyad, M. 2019. Potential effects of ursodeoxycholic acid on accelerating cutaneous wound healing. Plos One 14(12), e0226748.

Engwa, G.A. 2018. Free radicals and the role of plant phytochemicals as antioxidants against oxidative stress-related diseases. Phytochemicals: source of antioxidants and role in disease prevention. BoD–Books on Demand 7(1), 49–74.

Fasihnia, S.H., Peighambardoust, S.H., Peighambardoust, S.J., Oromiehie, A., Soltanzadeh, M. and Peressini, D. 2020. Migration analysis, antioxidant, and mechanical characterization of polypropylene-based active food packaging films loaded with BHA, BHT, and TBHQ. J. Food Sci. 85(8), 2317–2328.

Fikri, F., Rahmaningtyas, I.H. and Purnama, M.T.E. 2020. Antibacterial activity of *Annona muricata* Linn ethanolic extraction against *Pseudomonas aeruginosa in vitro*. Indian Vet. J. 97(4), 38–40.

Gupta, S., Mujawdiya, P., Maheshwari, G. and Sagar, S. 2022. Dynamic role of oxygen in wound healing: a microbial, immunological, and biochemical perspective. Arch. Razi. Ins. 77(2), 513.

Hamid, I.S., Aksono, E.B., Sukmanadi, M. and Purnama, M.T.E. 2018. Antiangiogenesis activity test of tin leaf (*Ficus carica L.*) on the number of blood vessels and VEGF expression of chorioallantoic membrane of embryonated chicken eggs. Eur. J. Oncol. Pharm. 1(4), e00007.

Haryanti, S., Widiyastuti, Y. and Rahmawati, N. 2018. Cytotoxic and MMPs inhibitory activities of Sappan Wood (*Caesalpinia sappan* L.): various extracts on 4T1 breast cancer cell line. Health Sci. J. Indones. 9(1), 51–56.

Hisyam, M.A.M., Yuniarti, W.M., Theresia, S.W., Yudaniayanti, I.S., Lukiswanto, B.S., Sudjarwo, S.A. and Widiyatno, T.V. 2023. The Efficacy of Madeira Vine Leaf Extract Combined with Turmeric Rhizome Extract Gel Topically on Second-Degree Burn Wound in Albino Rats. J. Med. Vet. 6(3), 402–409.

Hosseinzadegan, H. and Tafti, D.K. 2017. Mechanisms of platelet activation, adhesion and aggregation. Thromb. Haemost. Res. 1(2), 1008.

Kartikasari, A.M., Dewi, C.M.S., Listyasari, N., Pratama, A.R. and Purnama, M.T.E. 2020. Effect of tadpole serum on thyroid hormones and cytotoxic T-cell activity in Wistar rats: a model of skin cancer. Indian Vet. J. 97(6), 34–37.

Lesmana, M.A., Amri, I.A., Cahyanissa, A.R., Geoputri, K.M., Anisa, A.K. and Hardian, A.B. 2023. Efficacy of oregano extract ointment on fibroblast cells and epidermis in albino rats with excisional wound model. J. Med. Vet. 6(2), 262–270.

Medel-Marabolí, M., Romero, J.L., Obreque-Slier, E., Contreras, A. and Peña-Neira, A. 2017. Effect of a commercial tannin on the sensorial temporality of astringency. Food Res. Int. 102(1), 341–347.

Panggabean, R.T., Sudjarwo, S.A., Ma'ruf, A., Widiyatno, T.V., Yudaniayanti, I.S., Kurnijasanti, R. and Khairullah, A.R. 2023. Efficacy of *Sauropus androgynous* leaves extract gel on burn wound healing in albino rats. J. Med. Vet. 6(3), 418–426.

Pitaloka, R.P., Alipin, K., Syamsunarno, M.R.A.A., Utama, G.L., Panigoro, R. and Safitri, R. 2022. Sappan wood ethanol extract (*Caesalpinia sappan* L.) As adjuvant and substitute of iron chelator in acute iron overload rat model. Adv. Anim. Vet. Sci. 10(12), 2589–2595.

Prastika, D.D., Setiawan, B., Saputro, A.L., Yudaniayanti, I.S., Wibawati, P.A. and Fikri, F. 2020. Effect of shrimp chitosan topically on collagen density as excision wound healing parameter in albino rats. J. Med. Vet. 3(1), 101–107.

Punjataewakupt, A., Napavichayanun, S. and Aramwit, P. 2019. The downside of antimicrobial agents for wound healing. Eur. J. Clin. Microbiol. Infect. Dis. 38(1), 39–54.

Ramirez-Pedraza, M. and Fernández, M. 2019. Interplay between macrophages and angiogenesis: a double-edged sword in liver disease. Front. Immunol. 10(1), 2882.

Rani, C.A.M., Safira, A., Suryadiningrat, M., Fikri, F., Wardhana, D.K. and Purnama, M.T.E. 2022. Characterization of Tilapia collagen-loaded chitosan nanofibers synthesized by electrospinning method for wound dressing. IOP Conf. Ser. Earth Environ. Sci. 1036(1), 012034.

Rodrigues, M., Kosaric, N., Bonham, C.A. and Gurtner, G.C. 2019. Wound healing: a cellular perspective. Physiol. Rev. 99(1), 665–706.

Shin, K.O., Choe, S.J., Uchida, Y., Kim, I., Jeong, Y. and Park, K. 2018. Ginsenoside Rb1 enhances keratinocyte migration by a sphingosine-1-phosphate-dependent mechanism. J. Med. Food 21(11), 1129–1136.

Solikhah, T., Rani, C.A.M., Septiani, M., Putra, Y.A.S., Rachmah, Q., Solikhah, G.P., Agustono, B., Yunita, M.N. and Elziyad Purnama, M.T. 2022. Antidiabetic of *Hylocereus polyrhizus* peel ethanolic extract on alloxan induced diabetic mice. Iraqi J. Vet. Sci. 36(3), 797–802.

Spielman, A.F., Griffin, M.F., Parker, J., Cotterell, A.C., Wan, D.C. and Longaker, M.T. 2023. Beyond the scar: a basic science review of wound remodeling. Adv. Wound Care 12(2), 57–67.

Stanley, B.J. 2017. Negative pressure wound therapy. Vet. Clin. Small Anim. Pract. 47(6), 1203–1220.

Triana, N.M., Wilujeng, E., Putri, M.W.H., Yuda, D.M.P., Hardiono, A.L., Purnama, M.T.E. and Fikri, F. 2020. Antiproliferation effects of *Glycine max Linn* ethanolic extract on induced mammary gland carcinoma in albino rats. IOP Conf. Ser. Earth Environ. Sci. 441(1), 012103.

Tukiran, T., Salma, N.A., Sutoyo, S. and Sabila, F.I. 2023. Anti-arthritic activity of combination of *Caesalpinia sappan* and *Zingiber officinale* extracts in complete freund's adjuvant-induced arthritic in rats. Trop. J. Nat. Prod. Res. 7(1), 11.

Vij, T., Anil, P.P., Shams, R., Dash, K.K., Kalsi, R., Pandey, V.K., Harsányi, E., Kovács, B. and Shaikh, A.M. 2023. A comprehensive review on bioactive compounds found in *Caesalpinia sappan*. Mol. 28(17), 6247.

Wlaschek, M., Singh, K., Sindrilaru, A., Crisan, D. and Scharffetter-Kochanek, K. 2019. Iron and iron-dependent reactive oxygen species in the regulation of macrophages and fibroblasts in non-healing chronic wounds. Free Radic. Biol. Med. 133(1), 262–275.

Yuniarti, W.M., Primarizky, H. and Lukiswanto, B.S. 2018. The activity of pomegranate extract standardized 40% ellagic acid during the healing process of incision wounds in albino rats (*Rattus norvegicus*). Vet. World 11(3), 321.

Zeb, A. 2020. Concept, mechanism, and applications of phenolic antioxidants in foods. J. Food. Biochem. 44(9), e13394.

Zhu, J. 2018. T helper cell differentiation, heterogeneity, and plasticity. Cold Spring Harb. Perspect. Biol. 10(10), a030338.

Zulueta, M.M.L., Chyan, C.L. and Hung, S.C. 2018. Structural analysis of synthetic heparan sulfate oligosaccharides with fibroblast growth factors and heparin-binding hemagglutinin. Curr. Opin. Struct. Biol. 50(1), 126–133.